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April 28, 2005
[1057783.doc]
PS/UF

File No.:	*Our Reference:*	*Owner/Applicant:*
PCT/EP2004/006698	AX02A02/P-WO	Axiogenesis AG et al.

International application "Tissue modeling in embryonic stem (ES) cell system"

This is in response to the Written Opinion drawn up in accordance with Rule 43*bis*.1 PCT issued with the international search report on February 1, 2005, and to be considered to be a Written Opinion of the International Preliminary Examining Authority ("IPEA").

Herewith, a demand for international preliminary examination is made according to the enclosed form PCT/IPEA/401. The prescribed fees in the amount of € 1659.00 are to be debited from our deposit account No. 2800 0980; see also the enclosed Annex to form PCT/IPEA/401.

Furthermore, please find enclosed a new set of claims, which should form the basis for the international preliminary examination. In addition, a substitute page 30 of the description and a substitute sheet for figure 3A are enclosed herewith.

In the following, we would like to comment on the observations raised in the Written Opinion. In doing so, we will refer to the enclosed new set of claims.

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1. Amendments to the claims, the description and the figures

1.1 Claims 1 to 14 correspond to original claims 1 to 14.

1.2 Amended claim 15 corresponds to original claim 15 with the amendment that the features imported by claim 15 due to its reference to claim 1 have been incorporated for the sake of clarity. In particular, it has been clarified that in accordance with the method of claim 1 the claimed co-culture comprises cells which are capable of integration and alignment into tissue or tissue-like structures.

1.3 Amended claim 16 corresponds to original claim 16 with a minor editorial amendment.

1.4 Claims 17-44 correspond to claims original claims 17-44.

1.5 Amended claim 45 corresponds to original claim 45 with the amendment that an inconsistency in the dependency on the method claims has been corrected.

1.6 Amended claims 46 and 47 correspond to original claims 46 and 47 except that due to the amendment effected to the dependency in claim 45 the tissue referred to in claims 46 and 47 has now been alternatively been characterized by reference to claim 16.

1.7 Amended claim 48 corresponds to original claim 48 with the same amendment to the tissue recited in the claimed composition as has been effected for claims 46 and 47; see section 1.6, supra. Furthermore, the recombinant nucleic acid molecules as present in the composition of claim 48 have been characterized more clearly by incorporating the corresponding characterizing feature of said recombinant nucleic acid molecules recited in claim 21.

1.8 Claim 49 corresponds to original claim 49.

1.9 Amended claim 50 corresponds to original claim 50 with the same amendment with respect to the tissue recited in the claim as effected in amended claims 46, 47 and 48; see section 1.6, supra.

1.10 Amended claim 51 corresponds to original claim 51 with the amendment that the term "cardiac, fibroblast and/or endothelium-specific regulatory sequences" has been replaced with the term "cardiac, and fibroblast and/or endothelium-specific regulatory sequences"

in order to indicate more clearly that the recited recombinant nucleic acid molecule comprises inter alia at least a cardiac-specific regulatory sequence and in addition thereto a fibroblast and/or endothelium-specific regulatory sequence. This amendment is supported by the description of the present application; see, for example, page 4, lines 30-33 and the corresponding embodiment described at page 29, line 19 to page 30, line 18.

Furthermore, in feature (e) the term "cardiomyocytes, fibroblasts and/or endothelial cells" has been replaced with the term "cardiomyocytes, and said fibroblasts and/or endothelial cells", accordingly.

1.11 Claims 52-58 correspond to original claims 52 to 58.

1.12 Amended claim 59 corresponds to original claim 59, wherein the recited cells alternatively have been characterized by reference to claim 58. This amendment is supported by the description of the present application, for example at page 7, first full paragraph and page 31, line 19ff. In addition, reference to the tissue in claim 59 has been amended for the same reasons as discussed for claims 46 and 47; see section 1.6, supra.

1.13 Claims 60 and 61 correspond to original claims 60 and 61.

1.14 Amended claim 62 corresponds to original claim 62, wherein the same amendments have been effected as for claim 59; see section 1.12, supra.

1.15 Claims 63-80 correspond to original claims 63 to 80.

1.16 Amended claim 81 corresponds to original claim 81 with the same amendments for the recited cells and tissue, respectively, as discussed for claim 59; see section 1.12, supra.

1.17 Furthermore, a clerical error has been corrected in claims 26 and 54, i.e. the term "catherin" has been corrected to "cadherin". The same correction has been effected in substitute page 30 of the description and in figure 3A.

It is respectfully submitted that the effected amendments do not introduce new matter but merely have been effected in order to more clearly characterize the claimed subject matter or to correct obvious errors.

2. Clarity (Article 6 PCT)

2.1 Claims 15 and 16, 57 and 58

In the first paragraph of item VIII of the Written Opinion it is observed that the subject matter of claims 15 and 16, 57 and 58 is defined by technical features which have to be construed from the respective preceding method claims and, according to the Examiner, could possibly render the claims unclear.

However, as already acknowledged in the Written Opinion, the claimed products impart the properties, i.e. technical features as defined in the claims they are dependent on. For example, claim 15 relates to a co-culture of cells defined in anyone of claims 1 to 14, i.e. the culture of an embryonic stem (ES) cell-derived first cell type and an embryonic cell of a second cell type as mentioned in claim 1, and which are further defined in dependent claims 2 to 12, optionally comprising a further embryonic or ES cell-derived third cell type as mentioned in claim 13. Please note that each of the dependent claims directly or indirectly further defines said first, second and third cell type, respectively.

Therefore, the person skilled in the art will immediately and unambiguously recognize the subject matter for which protection is sought in claim 15.

The same line of arguments mutatis mutandis applies to claims 57 and 58.

Nevertheless, applicant amended claim 15 in order to recite the technical features as defined in claim 1. Furthermore, due to the amendment to claim 51, i.e. the clarification that the recombinant nucleic acid molecule comprises at least two different cell-specific regulatory sequences, also dependent claims 57 and 58 have been defined more clearly and thus now unambiguously define the subject matter for which protection is sought.

Accordingly, at least for the amended claims set the objections as set forth in the Written Opinion do not apply.

As a side remark it is noted that the applicant of course could also have drafted the embodiments encompassed by the subject matter of claims 15, 16, 57 and 58 as a separate set of independent and dependent claims. However, this measure would have indeed resulted in an excessive number of more than 100 claims. Instead, the applicant has aimed at keeping the claims clear and concise, which should be acknowledged by the Examiner.

For the above reasons, it is submitted that the contested claims meet the requirements of Article 6 PCT.

2.2 Claims 40 to 50, 59, 60, 62 to 75, 80 and 81

In the second paragraph of item VIII of the Written Opinion, objections are raised against the multiple dependency in claims 40 to 50, 59, 60, 62 to 75, 80 and 81 because of Rule 6.4 PCT.

However, it is respectfully submitted that this objection is moot since the requirements for the manner of claiming is a matter of National Law; see also the last sentence of Rule 6.4(a) PCT. For example, there is no corresponding provision in the EPC. To the contrary, multiple dependencies of claims as used in the present claims set is explicitly allowable in the EPO; see Rule 29(4) EPC.

For this reason, it is requested that the objection against the multiple dependency in the claims of the present application is not further pursued.

2.3 Number of claims

In the fourth paragraph of item VIII of the Written Opinion the International Searching Authority objects to the total number and number of independent claims under Rule 6.1(a) PCT.

However, as will also be evident from the explanations given below, the present invention is based on the novel and surprising finding that the layout of tissues constituting an adult organism, i.e. the interconnections and architectures of different cell types can be engineered in vitro. Hence, the present invention for the first time provides a reliable and cost-efficient source for human tissue and tissue-like structures that can be used for various applications, for example transplantation, toxicity tests, drug screening and of course basic developmental research. Thus, due to the generality of the present invention, there are several kinds of aspects the applicant must be allowed to claim. Furthermore, in view of the manifold techniques in molecular and cell biology various embodiments can be performed by the person skilled in the art in order to put the invention into practice. Accordingly, applicant must also be allowed to claim these various embodiments.

In summary, applicant holds the view that the number of claims simply reflects the nature and field of the invention, and is commensurate with the contribution to the art by making the teachings of the present invention available to the public.

Therefore, it is respectfully submitted that the number of claims is commensurate with the nature of the invention and the technology at stake.

3. Sufficiency (Article 5 PCT)

3.1 The present invention is generally applicable and enabled

In the last section of item VIII of the Written Opinion the International Searching Authority seems to object to the breadth of the claims since they cover any tissue or organ produced in accordance with the methods of the present invention, while according to the Authority only the modeling of cardiac tissue is exemplified in the present application. More specifically it is argued that the person skilled in the art would find no guidance in the present application as to suitable cell types to select as the second or further cell types to support the first cell type in order to arrive at, for example, pancreatic tissue or neural tissue.

It is respectfully submitted that the concerns of the Authority are unfounded.

The present invention provides novel and generally applicable methods of tissue modeling. As noted by the Authority, these methods have been exemplified for modeling and obtaining cardiac tissue.

However, this does not mean that the method of the present invention may not be applied to other tissue merely because of the absence of further examples.

First of all, there is no provision in, for example, the EPC that each and every embodiment falling within a generic claim has to be described in detail, let alone exemplified in the application. The same holds true for the PCT, where Article 5 PCT requires that the description shall disclose the invention in a manner sufficiently clear and complete for it to be carried out by a person skilled in the art. As set out in Rule 5.1(a)(v) PCT, the description may only set forth at least the best mode contemplated by the applicant for carrying out the invention or, where the best mode is not required, any mode.

In the present application, the applicant provided several examples which at the filing date of the present application may even be considered to provide the best mode of how to perform the claimed invention. Thus, the present application has met the requirements of Article 5 and Rule 5.1(a)(v) PCT.

Furthermore, the person skilled in the art when reading the description and the examples of the present application would be well aware how to apply the methods of the present invention to tissue other than cardiac tissue. In this respect, the person skilled in the art has certainly not to be taught fundamental aspects of cell biology but of course must be assumed to be skilled in the relevant field.

For example, the person skilled in the art is well aware of the structure of other tissue, for example neural tissue, which consists of nerve cells, called neurons, and supporting cells such as oligodendrocytes and astrocytes; see also the description of the present application at page 26, lines 12-15.

Having this knowledge, the person skilled in the art considering the method of claim 1 for modeling and/or obtaining neural tissue would be taught by the present application to culture ES cell-derived nerve cells, i.e. neurons as said first cell type in the presence of at least one embryonic supporting cell as the second cell type, for example oligodendrocytes and/or astrocytes. If using both oligodendrocytes and astrocytes also said third cell type is present.

With respect to the provision of ES cell-derived in vitro differentiated neural cells, the present application provides sufficient guidance how to arrive at such cells; see the description, for example at page 17, lines 4 and 5 as well as the paragraph from lines 12-18; page 22, lines 6-14 and page 30, lines 20-32.

Furthermore, sources and methods of preparing embryonic oligodendrocytes suitable for the purposes of the present invention are well-known to the person skilled in the art; see, for example, Gumpel et al., Ann. N. Y. Acad. Sci. 495 (1987), 71-85. The same applies to embryonic astrocytes and Schwann cells; see, for example, Kikuchi et al., Neurol. Med. Chir. 33 (1993), 682-690.

Thus, the person skilled in the art would find in the application sufficient disclosure and guidance as to how to select the suitable cell types for said first, second and optionally third cell type to support said first cell type which characterizes the target tissue.

3.2 The present invention has several benefits

Furthermore, also the benefits of the method of tissue modeling when applied to, for example, neural tissue are clear. For example, successful transplantation of neural cells requires a favorable environment in order to allow regeneration; see, for example, Kikuchi et al. (1993), supra.

In this respect, the benefit of the present invention is that according to the method of tissue modeling of the present invention neural tissue can be preformed in its favorable environment in vitro. This so-to-say preformed tissue can then advantageously be used for, e.g., transplantation purposes or in vitro studies of, for example, potential drugs. The latter embodiment is of benefit since on the one hand the tissue obtained in accordance with the method of the present invention comes closer to the in vivo situation as compared to, for example, a neuronal cell line only and on the other hand can be more easily and reliably be prepared than, for example, grafts from animals. Furthermore, the method of the present invention allows the modeling and production of human tissue.

From the foregoing it is evident that the methods of tissue modeling and production of the present invention have several benefits not only for cardiac tissue but also for other tissue such as neural tissue.

Given the fact that the methods of the present invention can indeed be generally applied to any given tissue and in the absence of evidence that the methods of the present invention would not work for certain types of tissue, the applicant should be given the benefit of doubt.

For the above reasons it is respectfully requested that the objections under Article 5 PCT be withdrawn.

4. Novelty (Article 33(2) PCT)

4.1 International application WO03/010303 (D1) and Mummery et al. (D1a)

In the first paragraph of item V of the Written Opinion it is argued that the subject matter of claims 1, 9, 10, 12, 15-17, 45-48, 50, 80 and 81 is allegedly not novel over D1 and D1a, respectively, since according to the Authority they teach the differentiation of embryonic stem cells by co-culture with visceral endoderm END-2 cells.

However, it is respectfully submitted that the assessment of the teaching of D1 and D1a in relation to the claimed invention is not entirely correct.

First of all, as already stated in the title of D1 and more particularly in that of D1a, these documents teach a method of inducing differentiation of stem cells into one particular cell type, i.e. muscle cells or vascular endothelial cells; see D1, for example, at the abstract, at page 1, 1st paragraph, and more specifically at the paragraph bridging pages 8

and 9 of D1, wherein the term "inducing differentiation of a stem cell" as used in the claimed methods has been defined. Consistent with that, the examples of D1 teach the differentiation of human embryonic stem cells into cardiomyocytes or visceral endoderm cells, respectively.

With respect to the cited co-culture with visceral endoderm END-2 cells, it is clear from the description and the examples that the END-2 cells are used as a kind of feeder cells which induce the stem cells to differentiate into one particular phenotype; see D1 at page 8, last full paragraph and the paragraph bridging pages 11 and 12. This is more clearly stated in D1a, the corresponding article to D1, wherein the "co-culture" is explained as follows:

> "... END-2 cell cultures treated for 3 hours with mitomycin C (mit.C; 10 µg/ml)[I] replaced mouse embryonic fibroblasts (MEFs) as feeders for hES cells"; see D1a at page 2734, left column, 2nd sentence of the method section "Cell Culture", (emphasis added).

In contrast, the method of the invention as claimed in claim 1 relates to a method of modeling and/or obtaining tissue or tissue-like structures comprising culturing an embryonic stem cell-derived first cell type in the presence of at least one embryonic second cell type, wherein said at least two cell types are allowed to integrate and align into tissue or tissue-like structures.

Thus, the method of claim 1 does not relate to the induction of differentiation of ES cells to cardiomyocytes but to the integration and alignment of in vitro differentiated cells such as cardiomyocytes with a second cell type, for example fibroblasts. As a result, always tissue and tissue-like structures are obtained, which consist of at least two cell types. In contrast, D1 and D1a disclose a method for in vitro differentiation of ES cells into one particular cell type, i.e. cardiomyocytes, and wherein the "supporting" END-2 cells are not allowed to integrate and align with the cardiomyocytes.

For the above reasons, the subject matter of claim 1 is novel over D1 and D1a.

The same mutatis mutandis applies to claims 9, 10, 12, 15-17, 45-48, 50, 80 and 81 which either directly or indirectly import the mentioned features as characterized in claim 1.

4.2 International application WO02/051987 (D2)

In the second paragraph of item V of the Written Opinion, the present Authority argues that the subject matter of claims 18-20, 45-58, 80 and 81 might be anticipated by D2, since the examples of D2 teach the transfection of ES cells with resistance and reporter genes under the control of a cardiac-specific promoter and selection of differentiated cardiomyocytes as well as their application in screening and therapy.

However, this observation does not anticipate the subject matter of the contested claims.

D2 is an international application by the present applicant and indeed discloses preferred embodiments of certain features to be used in accordance with the invention of the present application. In particular, D2 discloses an advantageous system for selecting ES cell-derived in vitro differentiated cells.

However, D2 does not disclose a method for improving cardiac function in a mammal in accordance with claim 18 of the present application, wherein in vitro differentiated cardiomyocytes are co-transplanted with embryonic or ES cell-derived fibroblasts as recited in feature (c) of claim 18 and feature (e) of claim 51. In this respect, due to the term "co-transplanting" said feature (c) and (e), respectively, has to be interpreted to mean that said cardiomyocytes are co-transplanted with fibroblasts and/or endothelial cells since otherwise the term "co-transplanting" would not make sense. Accordingly, the method of claim 18 and 51, respectively, does not encompass a method of transplanting cardiomyocytes only as disclosed in the examples of D2.

Nevertheless, as discussed in section 1.10, supra, applicant amended claim 51 in order to more clearly indicate the gist of the present invention, i.e. the co-differentiation of at least two cell types into tissue-like structures. Accordingly, the observation of the Authority that the wording in original claims 51-53 includes the case where only cardiomyocytes are present at least does not apply to amended claim 51 and its dependent claims 52 and 53.

As mentioned, D2 does not disclose the novel approach of co-differentiation and co-transplanting of cells or tissue of at least two cell types.

Likewise, D2 does not disclose tissue of claim 45, in particular as amended, comprising said at least first and second cell types as defined in the preceding claims.

D2 also does not disclose a vector or a composition of vectors comprising recombinant nucleic acid molecules which altogether confer at least two different cell types to in vitro differentiating ES cells transfected with said recombinant nucleic acid molecules.

Therefore, the subject matter of claims 18-20 and 45-58 is novel over D2.

The same mutatis mutandis applies to claims 80 and 81.

4.3 US patent 5,733,727 (D3)

In the third paragraph of item V of the Written Opinion the present Authority inter alia also argues that document D3, in particular example 4, could possibly destroy the novelty of claims 18-20, 45-58, 60-75, 80 and 81.

However, this objection is in error for similar reasons as the Authority's assessment of D2 in relation to the claimed invention.

D3, in particular example 4, merely discloses a method of obtaining a population of cardiomyocytes by transfecting ES cells with a marker gene enabling selection of a cardiomyocyte cell lineage; see, for example, claim 1 and the description of example 4, in column 12, lines 42-51 and in column 13, lines 26-31.

However, D3 neither teaches nor suggests co-culturing or co-transplanting of at least two different cell types, for example cardiomyocytes and fibroblasts. Consequently, D3 does also not disclose corresponding co-cultures, tissue, organs etc. and uses thereof.

Accordingly, the contested claims are novel over D3.

4.4 Publication by Müller et al. (D4)

D4 relates to a method for the generation and selection of ventricular-like cardiomyocytes from ES cells in vitro. Hence, similar to D2 and D3, also D4 does not disclose the use of a second cell type in any way in order to obtain corresponding tissue or tissue-like structures or to co-transplant, for example, cardiomyocytes with a second cell type like fibroblasts.

To the contrary, the entire publication including the discussion section is clearly confined to the possibility of differentiating cardiomyocytes from human ES cells and their use in drug discovery and cell-mediated gene therapy without mentioning at all the need or the advantage of using a second cell type in order to improve these applications; see, for example, the discussion of D4 at page 2547, left column, last full paragraph.

For this reason, claims 18-20, 45-58, 62-75, 80 and 81 are also novel over D4.

4.5 Publication by Spielmann et al. (D5)

In the fourth paragraph of item V of the Written Opinion, the present Authority considers that the use of ES cells transfected with a reporter gene under the control of a cardiac-specific promoter and of cardiomyocytes derived from ES cells in toxicity tests in vitro would anticipate the subject matter of claims 45-49, 57, 58, 62-75, 80 and 81.

However, also D5 is confined to the use of ES cells differentiated into cardiomyocytes only and does not disclose or suggest the need or advantage of using tissue or tissue-like structures comprised of at least two different cell types for the described embryonic stem cell test.

Accordingly, the subject matter of the objected claims is novel over D5.

4.6 Abstract by Kettenhofen et al. (D6)

Similar do documents D2 to D5 also D6 discloses a selection system for in vitro differentiated cardiomyocytes and their use for screening purposes for developmental toxicity.

However, D6 fails to teach or suggest a tissue or cell aggregate comprising at least two different cell types, for example, cardiomyocytes as the first cell type and fibroblasts as the second cell type as claimed in claim 45.

Accordingly, D6 does not anticipate the subject matter of claim 45. The same mutatis mutandis applies to dependent claims 46-49 as well as to claims 57, 58, 62-75, 80 and 81.

5. Inventive Step (Article 33(3) PCT))

5.1 The problem underlying the present invention and its solution

The problem underlying the invention claimed in the present application is the provision of tissue and tissue-like structures derived from embryonic stem (ES) cells, which as much as possible resemble native human tissue and thus can advantageously be used for transplantation purposes as well as drug screening and toxicity testing.

This problem has been solved by the novel and inventive unifying concept to co-culture or co-transplant an embryonic stem (ES) cell-derived in vitro differentiated first cell type together with at least one further embryonic or ES cell-derived in vitro differentiated cell of a second cell type, wherein said at least two cell types are capable of integration and alignment into tissue or tissue-like structures.

As taught in the application and demonstrated in the examples, this novel and inventive concept of providing in vitro generated de novo tissue can be put into practice in various ways, i.e. as characterized in the embodiments of the independent claims.

Furthermore, the examples of the present application demonstrate that the above-defined problem has been solved in accordance with the different embodiments claimed.

5.2 The closest prior art

According to the present Authority, documents D1/D1a and D2, respectively, represent the closest prior art.

As already acknowledged by the present Authority, both documents only disclose culture and selection processes, respectively, for the provision of one particular in vitro differentiated cell type, i.e. cardiomyocytes.

As furthermore acknowledged, none of the prior art documents suggests to derive simultaneously several cell types from ES cells and to allow these cell types to support each other and to give rise to a functional tissue.

Accordingly, the present Authority already acknowledged novelty and inventive step for original claims 21-39 as well as for the cells and cell aggregates of claims 43 and 44, respectively.

It is respectfully submitted that the conclusion reached by this Authority with respect to claims 21-39 and 43 and 44 applies to the entire set of claims.

As has been demonstrated in section 4, supra, all claims are novel over the cited prior art. In this context, it is also explained that each embodiment carries the novel and inventive concept of paralleling two different cell types in order to generate tissue or tissue-like structures.

Furthermore, as discussed in section 3, supra, the claimed invention has general applicability and several benefits over the teaching in the prior art. Thus, the present invention provides tissue and tissue-like structures which are more suitable for transplantation purposes than one single in vitro differentiated cell type because it comes closer to the structure of the damaged tissue in the body. It is evident that such tissue and tissue-like structures are also more suitable for toxicity testing, in particular with respect to developmental toxicity.

In contrast, prior to the present invention, the focus in ES cell technology has always been the differentiation, selection and investigation into one particular cell type rather than approaching the problem of providing more complex tissue-like structures.

For the above reasons, inventive step for all claims should be acknowledged.

6. Requests

With the above explanations and the amendments to the claims, it is submitted that the applicant has met the requirements of the PCT. It is therefore requested that the Authority's objections be withdrawn and that a favorable IPER be issued.

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Enclosures
New set of claims 1-81 (in triplicate)
Substitute page 30 of the description (in triplicate)
Substitute figure 3A (in triplicate)
Form PCT/IPEA/401 including Annex

Claims

1. Method of modeling and/or obtaining tissue or tissue-like structures comprising culturing an embryonic stem (ES) cell-derived first cell type in the presence of at least one embryonic second cell type; and allowing integration and alignment of said at least two cell types into tissue or tissue-like structures.

2. The method of claim 1, wherein the ES cell of said ES cell-derived first cell type comprises a selectable marker operably linked to a first cell type-specific regulatory sequence specific for said first cell type.

3. The method of claim 2, wherein said selectable marker confers resistance to puromycin.

4. The method of any one of claims 1 to 3, wherein said ES cell of said ES cell-derived first cell type comprises a reporter gene operably linked to a cell type-specific regulatory sequence specific for said first cell type.

5. The method of claim 4, wherein said cell type-specific regulatory sequence of the reporter gene is substantially the same as said first cell type-specific regulatory sequence of the marker gene.

6. The method of claim 5, wherein said reporter is selected from different color versions of enhanced green fluorescent protein (EGFP).

7. The method of any one of claims 4 to 6, wherein said marker gene and said reporter gene are contained one the same recombinant nucleic acid molecule.

8. The method of claim 7, wherein said marker gene and said reporter gene are contained on the same cistron.

9. The method of any one of claims 1 to 8, wherein said first cell type is selected from the group consisting of neuronal cells, glial cells, cardiomyocytes, glucose-responsive insulin-secreting pancreatic beta cells, hepatocytes, astrocytes, oligodendrocytes,

chondrocytes, osteoblasts, retinal pigment epithelial cells, fibroblasts, keratinocytes, dendritic cells, hair follicle cells, renal duct epithelial cells, vascular endothelial cells, testicular progenitors, smooth and skeletal muscle cells.

10. The method of any one of claims 1 to 9, wherein said first cell type are cardiomyocytes.

11. The method of claim 10, wherein said first cell type-specific regulatory sequence is atrial and/or ventricular specific.

12. The method of claim 10 or 11, wherein said at least one embryonic second cell type are fibroblasts or endothelial cells.

13. The method of any one of claims 1 to 12, further comprising culturing said at least two cell types in the presence of an embryonic or embryonic stem (ES) cell-derived third cell type.

14. The method of claim 13, wherein said third cell type are endothelial cells or fibroblasts.

15. A co-culture of cells as defined in any one of claims 1 to 14, comprising at least cells of said first and second cell type under conditions, wherein said cells are capable of integrating and alignment into tissue or tissue-like structures.

16. A tissue obtainable by the method of any one of claims 1 to 14.

17. A method of improving tissue repair and/organ function in a mammal comprising the steps of:

(a) introducing a cellular inoculum comprising a co-culture of cells of claim 15 in which differentiation has been initiated or tissue of claim 16 to at least a portion of the previously damaged area of the tissue; and

(b) allowing said introduced cellular inoculum to engraft in situ as viable cells or tissue situated within the previously damaged area of the tissue, wherein the engraftment results in improved tissue and/or organ function in said mammal.

18. A method for improving cardiac function in a mammal after a myocardial infarct, said method comprising the steps of:

(a) culturing undifferentiated mammalian embryonic stem (ES) cells comprising a resistance gene and a reporter gene under the control of the same cardiac-specific promoter in vitro in a culture medium containing the selective agent for the resistance gene under conditions allowing differentiation of said ES cells into cardiomyocytes;

(b) isolating said differentiated cardiomyocytes and/or eliminating non-differentiated cells, optionally along with cells differentiating towards irrelevant cell types from said cardiomyocytes in the course of differentiation;

(c) subsequently co-transplanting said cardiomyocytes with embryonic or ES cell-derived fibroblasts to at least a portion of the previously infarcted area of the heart tissue; and

(d) allowing said introduced cellular inoculum to engraft in situ as viable cells situated within the previously infarcted area of the heart tissue, wherein the engraftment results in improved cardiac function in said mammal.

19. The method of claim 18, wherein said resistance gene and said reporter gene are contained in a bicistronic vector and separated by an IRES.

20. The method of claim 19, wherein said resistance gene confers resistance to puromycin, said marker is EGFP and said promoter is the cardiac αMHC promoter.

21. Method of modeling and/or obtaining tissue or tissue-like structures comprising the following steps:

(a) transfecting one or more multi- or pluripotent cells with recombinant nucleic acid molecules comprising a first and a second cell type-specific regulatory sequence operably linked to at least one selectable marker, wherein said second cell type is different from said first cell type;

(b) culturing the cells under conditions allowing differentiation of the cells; and

(c) isolating cells of at least two differentiated cell types and/or eliminating non-differentiated cells, optionally along with cells differentiating towards

irrelevant cell types from cell types of interest that activate the selectable marker in the course of differentiation.

22. The method of claim 21, further comprising transfecting said one or more cells with recombinant nucleic acid molecules comprising at least one further cell type-specific regulatory sequence operably linked to at least one selectable marker, wherein said at least one further cell type is different from said first and second cell type.

23. The method of claim 21 or 22, wherein said cells are embryonic stem (ES) or embryonic germ (EG) cells.

24. The method of any one of claims 21 to 23, wherein said recombinant nucleic acid molecules are comprised in the same vector or different vectors.

25. The method of any of claims 21 to 24, wherein said cell type is selected from the group consisting of neuronal cells, glial cells, cardiomyocytes, glucose-responsive insulin-secreting pancreatic beta cells, hepatocytes, astrocytes, oligodendrocytes, chondrocytes, osteoblasts, retinal pigment epithelial cells, fibroblasts, keratinocytes, dendritic cells, hair follicle cells, renal duct epithelial cells, vascular endothelial cells, testicular progenitors, smooth and skeletal muscle cells.

26. The method of claim 7 or 8, wherein said promoter is selected from the group consisting of αMHC, MLC2V, cadherin, Tie-2 and collagen promoter.

27. The method of any one of claims 21 to 26, wherein said one or more recombinant nucleic acid molecules are transfected concomitantly or subsequently into said one or more cells.

28. The method of any one of claims 21 to 26, wherein at least two different cells or clones thereof are transfected and selected, wherein said at least two different cells or cell clones contain recombinant nucleic acid molecules with different cell type specific regulatory sequences.

29. The method of claim 28, wherein said at least two different cells or cell clones are mixed at the initial stage of differentiation in order to allow formation of cell aggregates.

30. The method of claim 29, wherein said cell aggregates are chimeric embryoid bodies (EBs).

31. The method of any one of claims 21 to 30, wherein one of said cells or cell clones thereof is transfected and selected, wherein said cell or cell clone contains recombinant nucleic acid molecules with at least two different cell type-specific regulatory sequences.

32. The method of any one of claims 21 to 31, wherein at least two of said selectable marker operably linked to said different cell type specific regulatory sequences are identical.

33. The method of any one of claims 21 to 32, wherein at least one of said selectable marker is operably linked to said different cell type-specific regulatory sequences confers resistance to puromycin, bleomycin, hygromycin, methothrexate, or neomycin.

34. The method of any one of claims 21 to 33, wherein one or more of said recombinant nucleic acid molecules further comprise a reporter operably linked to said cell type-specific sequence.

35. The method of claim 34, wherein said reporter is selected from different color versions of enhanced green fluorescent protein (EGFP).

36. The method of claim 35, wherein EYFP (yellow), ECFP (blue) and/or hcRFP (red) are operably linked to different cell type-specific sequences.

37. The method of any one of claims 34 to 36, wherein said selectable marker and said reporter are expressed from a bicistronic vector.

38. The method of claim 37, further comprising one or more internal ribosomal entry sites (IRES), wherein said IRES separates said selectable marker and said reporter.

39. The method of any one of claims 21 to 38, further comprising allowing self-assembly of the different cell types.

40. The method of any of claims 1 to 14 or 21 to 39, further comprising analysing the physiological and/or developmental status of the cells or cell aggregate.

41. The method of claim 40, wherein the status is analyzed by monitoring the differentiation of electrical activity of the cells on an array.

42. The method of claim 41, wherein said status is analyzed by recording the extracellular field potentials with a microelectrode array (MEA).

43. A cell or cells obtainable by the method of any one of claims 21 to 42, wherein said cell or cells are capable of differentiating into at least two cell types.

44. A cell aggregate of at least two different cell types obtainable by the method of any one of claims 21 to 42.

45. A tissue obtainable by the method of any one of claims 21 to 42 or comprising cells of claim 43 or a cell aggregate of claim 44.

46. An organ comprising cells of claim 43, a cell aggregate of claim 44 or tissue of claim 16 or 45.

47. An implant or transplant comprising cells of claim 43, a cell aggregate of claim 44, a tissue of claim 16 or 45, or an organ of claim 46.

48. A composition of matter comprising recombinant nucleic acid molecules as defined in any one of claims 21 to 42, comprising at least a first and a second cell type-specific regulatory sequence operably linked to at least one selectable marker, wherein said

second cell type is different from said first cell type, cells of claim 43, a cell aggregate of claim 44, or a tissue of claim 16 or 45.

49. Use of the method of any one of claims 1 to 14 or 21 to 42 for analyzing early steps of tissue formation during embryonic development or the influence of factors and compounds on this process.

50. A method of treatment of damaged tissue or organs in a subject comprising implanting or transplanting to the subject in need thereof cells of claim 43, a cell aggregate of claim 44, a tissue of claim 16 or 45 or an organ of claim 46.

51. A method for improving cardiac function in a mammal after a myocardial infarct, said method comprising the steps of:

(a) transfecting mammalian embryonic stem (ES) cells with a recombinant nucleic acid molecule comprising a resistance gene under the control of cardiac, and fibroblast and/or endothelium-specific regulatory sequences, and optionally comprising one or more reporters under the same specific regulatory sequences;

(b) culturing said ES cells in vitro in a culture medium containing the selective agent for the resistance gene under conditions allowing differentiation of said ES cells into cardiomyocytes, fibroblasts and/or endothelial cells;

(c) eliminating from said differentiated cardiomyocytes, fibroblasts and/or endothelial cells non-differentiated cells, optionally along with cells differentiating towards irrelevant cell types; optionally

(d) allowing aligning and integration of said differentiating cardiomyocytes, fibroblasts and/or endothelial cells into cardiac-like tissue;

(e) subsequently co-transplanting said cardiomyocytes and said fibroblasts and/or endothelial cells or said tissue to at least a portion of the previously infarcted area of the heart tissue; and

(f) allowing said introduced cells or tissue to engraft in situ as viable cells situated within the previously infarcted area of the heart tissue, wherein the engraftment results in improved cardiac function in said mammal.

52. The method of claim 51, wherein said cardiomyocytes, fibroblasts and/or endothelial cells are derived from the same ES cell.

53. The method of claim 51, wherein said cardiomyocytes, fibroblasts and/or endothelial cells are derived from different ES cells.

54. The method of any of claims 51 to 53, wherein said cardiac-specific regulatory sequence is selected from promoters of αMHC, MLC22v, MLC1a, MLC2a and βMHC, said fibroblast-specific regulatory sequence is selected from promoters of Tie2, Tie1 and cadherin, and said endothelium-specific regulatory sequence is selected from promoters of collagen I promoters.

55. The method of any of claims 51 to 54, wherein said reporter for said cardiomyocytes, fibroblasts and/or endothelial cells is independently selected from the enhanced green fluorescent proteins ECFP (blue), EYFP (yellow) and hcRFP (red).

56. The method of any of claims 51 to 55, wherein said resistance gene and said reporter are separated by an internal ribosomal entry site (IRES).

57. A vector or a composition of vectors comprising the recombinant nucleic acid molecules as defined in any one of claims 51 to 56.

58. A cell or a plurality of cells comprising the vector or the composition of vectors of claim 57.

59. An array comprising a solid support and attached thereto or suspended thereon cells of claim 43 or 58, a cell aggregate of claim 44, or a tissue of claim 16 or 45.

60. The array of claim 59, which is a microelectrode array (MEA).

61. An apparatus for analyzing the array of claim 59 or 60.

62. A method for obtaining and/or profiling a test substance capable of influencing cell development and/or tissue structure formation comprising the steps:

(a) contacting a test sample comprising cells of claim 43 or 58, a cell aggregate of claim 44, a tissue of claim 16 or 45, an organ of claim 46 or an array of claim 59 or 60 with a test substance; and

(b) determining a phenotypic response in said test sample compared to a control sample, wherein a change in the phenotypic response in said test sample compared to the control sample is an indication that said test substance has an effect on cell development and/or tissue structure formation.

63. The method of claim 62, wherein said test sample is contacted with said test substance prior to, during or after said cell or cell aggregate passed through the method of any one of claims 1 to 14 or 21 to 42.

64. The method of claim 62 or 63, wherein said contacting step further includes contacting said test sample with at least one second test substance in the presence of said first test substance.

65. The method of any one of claims 62 to 64, wherein preferably in a first screen said test substance is comprised in and subjected as a collection of test substances.

66. The method of claim 65, wherein said collection of test substances has a diversity of about 10^3 to about 10^5.

67. The method of claim 66, wherein the diversity of said collection of test substances is successively reduced.

68. The method of any one of claims 61 to 67, which is performed on an array as defined in claim 59 or 60.

69. The method of any one of claims 61 to 68, wherein the phenotypic response comprises electrophysiological properties during the ongoing differentiation process.

70. The method of any one of claims 1 to 14, 21 to 42 or 62 to 69, wherein said one or more cells are genetically engineered to (over)express or inhibit the expression of a target gene.

71. The method of any one of claims 1 to 14, 21 to 42 or 62 to 70, wherein a compound known to activate or inhibit differentiation process and/or tissue structure formation is added to the culture medium.

72. The method of any one of claims 1 to 14, 21 to 42 or 62 to 71, wherein said one or more cells or tissue are contained in a container.

73. The method of any one of claims 1 to 14, 21 to 42 or 62 to 72, comprising taking 3 or more measurements, optionally at different positions within the container.

74 The method of any one of claims 72 or 73, wherein said container is a well in a microtiter plate.

75. The method of claim 74, wherein said microtiter plate is a 24-, 96-, 384- or 1586- well plate.

76. A method of manufacturing a drug comprising the steps of any one of claims 62 to 75.

77. A method of manufacturing an agent which supports wound healing and/or healing of damaged tissue comprising the steps of any one of claims 62 to 76.

78. The method of claim 76 or 77, further comprising modifying said substance to alter, eliminate and/or derivatize a portion thereof suspected causing toxicity, increasing bioavailability, solubility and/or half-life.

79. The method of any one of claims 76 to 78, further comprising mixing the substance isolated or modified with a pharmaceutically acceptable carrier.

80. A kit or composition useful for conducting a method of any one of claims 1 to 14, 21 to 42, 50 to 56 or 62 to 79, containing the vector or the composition of vectors of claim 57, a multi- or pluripotent cell, and optionally culture medium, recombinant nucleic acid molecules, or standard compounds.

81. Use of cells of claim 43 or 58, a cell aggregate of claim 44, a tissue of claim 16 or 45 or an organ of claim 46, the implant or transplant of claim 47, the vector or the composition of vectors of claim 57, the composition of claim 48, an array of claim 59 or 60 or an apparatus of claim 61 in drug discovery or pharmacokinetic or pharmacological profiling.

(e) subsequently co-transplanting said cardiomyocytes, fibroblasts and optionally endothelial cells or said tissue to at least a portion of the previously infarcted area of the heart tissue; and

(f) allowing said introduced cells or tissue to engraft in situ as viable cells situated within the previously infarcted area of the heart tissue, wherein the engraftment results in improved cardiac function in said mammal.

As mentioned before, said cardiomyocytes, fibroblasts and optionally endothelial cells are preferably derived from the same ES cell. However, cardiomyocytes, fibroblasts and optionally endothelial cells derived from different ES cells may be used as well. In those embodiments, said cardiac-specific regulatory sequence is preferably selected from promoters of αMHC, MLC2v, MLC1a, MLC2a and βMHC, said endothelium-specific regulatory sequence is preferably selected from promoters of Tie2, Tie1 and cadherin, and said fibroblast-specific regulatory sequence is preferably selected from promoters of collagen I; see supra. Similarly, said reporter for said cardiomyocytes, fibroblasts and optionally endothelial cells is independently preferably selected from the enhanced green fluorescent proteins ECFP (blue), EYFP (yellow) and hcRFP (red); see also Figure 3 and the examples. Said resistance gene and said reporter are preferably separated by an internal ribosomal entry site (IRES).

In another example, neuroepithelial cells are generated and used to augment or replace cells damaged by illness, autoimmune disorders, accidental damage, or genetic disorder. Mouse ES cells can be induced to differentiate in vitro with retinoic acid to form neuronal and glial precursors, positive for astrocyte (GFAP) or oligodendrocyte (O4) markers, then later into functional neurons (Fraichard et al., J. Cell Science 108 (1995), 3161-3188). Cells transplanted to adult brains were observed innervating the host striatum (Deacon et al., Exp. Neurology, 149 (1998), 28-41). Human and mouse EC cell lines can also differentiate into neurons. (Trojanowski et al., Exp. Neurology, 144 (1997), 92-97; Wojcik et al., Proc. Natl. Acad. Sci. USA, 90 (1993), 1305-1309). Transplantation of these neurons into rats subjected to cerebral ischemia promoted a degree of functional recovery (Borlongan et al., Exp. Neurology 149 (1998), 310-321). In accordance with the present invention, for this embodiment corresponding neuronal and glial specific promoters are used; see, e.g., Kawai et al., Biochim. Biophys. Acta 1625 (2003), 246-252, and Kugler et al., Gene Ther. 10 (2003), 337-347, for glial and neuronal specific promoters. Efficiency of embryoid body formation and hematopoietic development from embryonic stem cells in different culture systems is

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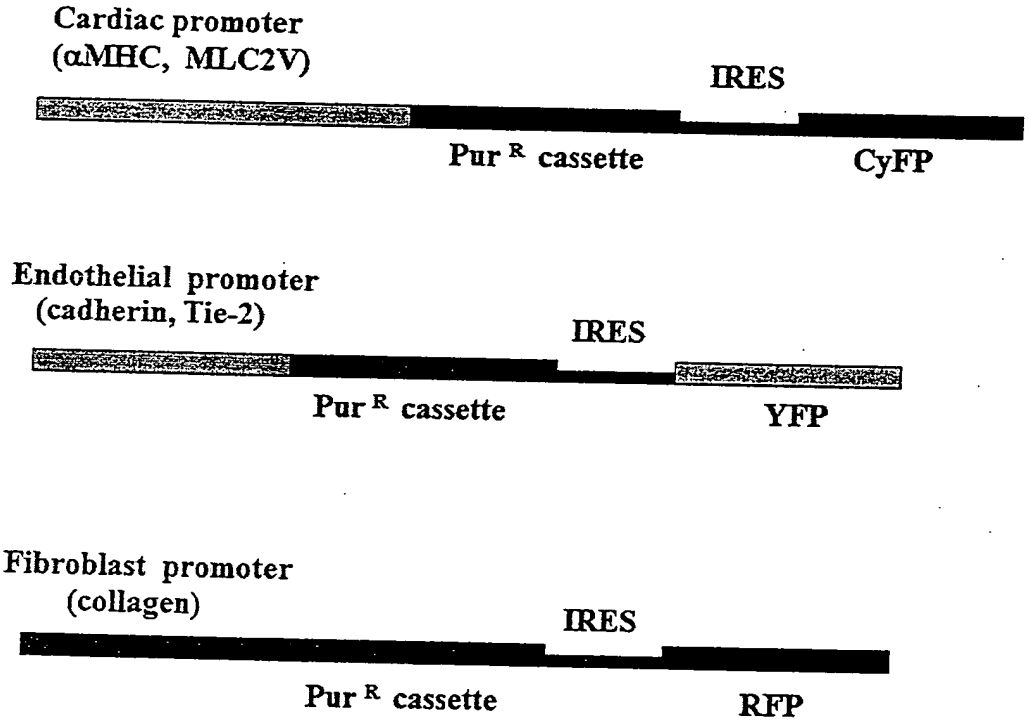
Cardiac promoter
(αMHC, MLC2V)
IRES
Pur R cassette
CyFP
Endothelial promoter
(cadherin, Tie-2)
IRES
Pur R cassette
YFP
Fibroblast promoter
(collagen)
IRES
Pur R cassette
RFP

Figure 3

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